LUD 5539 - JEL/MAS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Kohei MIYAZONO et al.
Serial No.	:	09/039,177
Filed	:	March 13, 1998
For	:	ISOLATED ALK-1 PROTEIN, NUCLEIC ACIDS ENCODING IT, AND USES THEREOF
Art Unit	:	1646
Examiner	:	D. Fitzgerald

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

DECLARATION OF
DR. ANITA B. ROBERTS AND
DR. ROBERT J. LECHLEIDER

Sir:

1. We, Anita B. Roberts and Robert J. Lechleider, are authors on Lechleider et al., "Serine Phosphorylation, Chromosomal Localization, and Transforming Growth Factor-β Signal Transduction by Human *hsp-1*", Journal of Biological Chemistry, 271:17617-17620 (1996) ("Lechleider et al.").

2. By 1998, we and others in the art believed that the phosphorylated molecules detected by Lechleider et al. and Yingling et al. "Mammalian dwarfins are phosphorylated in response to transforming growth factor β and are implicated in control of cell

growth", Proc. Natl. Acad. Sci., 93:8940-8944 (Aug. 1996), were not phosphorylated Smad-1 but rather other phosphorylated Smad molecules for the following reasons.

3. When Lechleider et al. and Yingling et al. were published in 1996 researchers in the field had not yet identified Smads other than Smad-1. Therefore, in 1996 we concluded that the antibodies that we were using, which were raised against a Smad-1 fusion protein were Smad-1 specific and were detecting a phosphorylated Smad-1. Thus, we reported in Lechleider et al. that Smad-1 was phosphorylated in response to TGF-β. We now conclude that molecules other than Smad-1 were phosphorylated in that assay.

4. Subsequent to the publication of Lechleider et al. and Yingling et al., other Smads were identified and found to have significant regions of homology with Smad-1. Those in this art came to appreciate that the antibodies used in the experiments reported in Lechleider et al. and Yingling et al. were not specific for Smad-1 due to their cross reactivity with the other homologous smads.

5. Between 1996 and 1998 additional experiments investigating the role of the various Smads in signaling pathways demonstrated that the phosphorylation of the Smads-1, 2, 3 and 5 were pathway restricted, i.e., Smad-2 and Smad-3 were phosphorylated and translocated to the nucleus after stimulation with TGF-β and Smad-1 and Smad 5 were phosphorylated and translocated after stimulation with BMPs. (See e.g., Heldin et al., Nature, 390:465-471(1997) page 466, right col., last paragraph through page 467, left col.)

6. Thus by 1998, we and others in the art believed that the phosphorylated molecules detected by Lechleider et al. and Yingling et al., were likely not phosphorylated Smad-1 but rather other phosphorylated Smad molecules.

7. We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both., under section 1001 of Title 18 of the United State Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

May 2, 2000
Date

Anita Roberts

2 May 2000
Date

Robert J. Lechleider

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HUMAN MUTATION

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Novel Missense and Frameshift Mutations in the Activin Receptor-Like Kinase-1 Gene in Hereditary Hemorrhagic Telangiectasia; Daniel J. Klaus, Carol J. Gallione, Kara Anthony, Eric Y. Yeh, Jing Yu, Andreas Lux, David W. Johnson, and Douglas A. Marchuk* *Department of Genetics, Duke University Medical Center*

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*Correspondence to: Douglas A. Marchuk, Department of Genetics, Duke University Medical, Box 3175, Durham, NC 27710; Fax: 919 681-9193;E-mail: douglas.marchuk@duke.edu

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Key Words: ALK-1, Activin-like kinase receptor, Hereditary Hemorrhagic Telangiectasia, HHT2

ABSTRACT

Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disorder characterized by multisystemic vascular dysplasia and recurrent hemorrhage. One of the causative genes is the activin receptor-like kinase-1 (ALK-1) gene located on chromosome 12q13. ALK-1 is an endothelial cell type I receptor for the TGF-β superfamily of ligands. As a number of mutations have been identified in the kinase domain of ALK-1, we initiated a mutation analysis specifically targeting the first four coding exons of ALK-1 in order to determine if mutations in the extracellular and transmembrane domains are also present in HHT. Six new mutations have been identified. Three frameshift mutations were identified in exons encoding the extracellular and transmembrane domains. These mutations would grossly truncate the ALK-1 protein and are thus classic null alleles. Three new missense mutations within the exons encoding the extracellular domain, in addition to two previously described missense mutations, are located at or near highly conserved cysteines. These mutations may disrupt intra- or inter-molecular disulfide bridges required for ligand binding. The combined data suggest that both severe and subtle changes in the ALK-1 amino acid sequence can lead to receptor dysfunction and result in the HHT disease phenotype. © 1998 Wiley-Liss, Inc.